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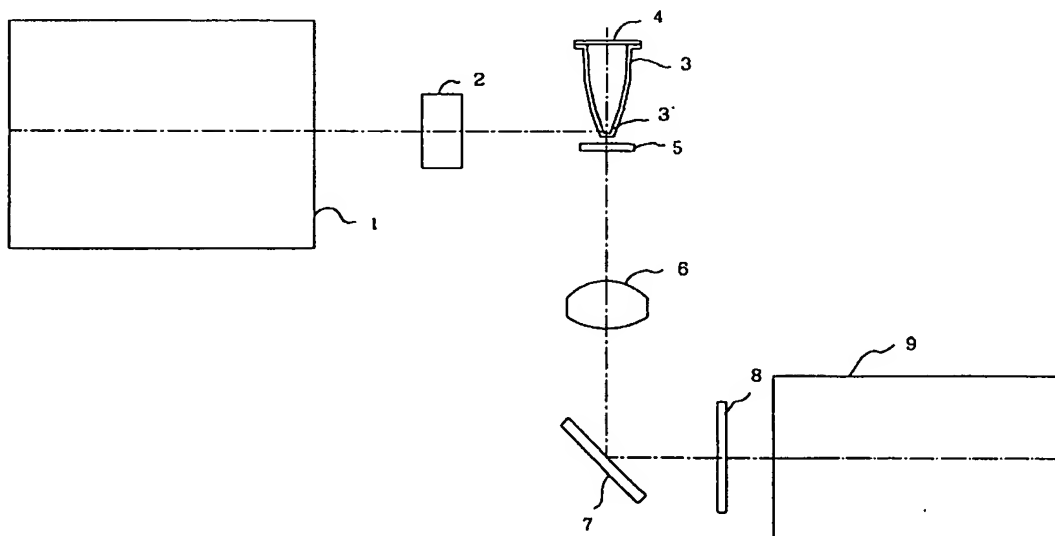
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(54) **Apparatus for imaging fluorescent particles**

(57) Fluorescent particles stained with a fluorescent dye are collected in the bottom portion of an imaging vessel (3) and imaged by a CCD camera (9). The fluorescent particles are illuminated by a beam of excitation

light (1), with only the bottom portion (3') or the vicinity thereof being illuminated to thereby reduce undesirable background light, resulting in the obtaining of images having improved contrast.

**FIG. 1**



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## Description

[0001] This invention relates to an apparatus for imaging fluorescent particles, and more particularly to an apparatus for imaging fluorescent particles such as leukocytes or the like stained with a fluorescent dye.

[0002] In the medical field, platelet preparation and erythrocyte preparation are produced by extracting platelets and erythrocytes from whole blood. These platelet and erythrocyte preparations are each used for blood transfusions, and it is undesirable for either preparation to contain leukocytes. It is therefore important to be able to know how many leukocytes the preparations contain. Conventionally this is done by placing a sample platelet preparation in a NAGEOTTE chamber, staining with a fluorescent dye, projecting an excitation light onto the sample and counting leukocytes via a microscope. Specifically, a 50 microliter sample is taken from a 200 or 400 milliliter bag of platelet preparation, the leukocytes in the sample are counted and converted to a leukocytes count for the whole bag. This is a tiring, inefficient, time-consuming task that has to be done by skilled personnel.

[0003] An apparatus has been proposed to enable leukocytes to be counted, instead, by staining the leukocytes with a fluorescent dye, illuminating the sample with an excitation light having predetermined wavelengths, using a CCD camera or the like to image the sample and then analyzing the images to obtain a count of the leukocytes. However, the solution containing the stained leukocytes also contains fluorescent dye that also emits fluorescent light. Thus, since not only the stained leukocytes but also the fluorescent dye itself is excited by the excitation light, there is a marked decrease in the contrast of the leukocytes that it is desired to observe or image. In some cases, the contrast may worsen to the point that the leukocyte images become so buried in the background that they cannot be picked out, making it impossible to count the leukocytes.

[0004] An object of the present invention is to provide an apparatus for imaging fluorescent particles that enables the fluorescent particles to be well imaged by reducing the effect of background light.

[0005] To attain the above object, the present invention provides an apparatus for imaging fluorescent particles stained with a fluorescent dye, comprising an imaging vessel for collecting and accommodating the fluorescent particles in a bottom portion thereof, means for generating an excitation light for exciting the fluorescent particles, illumination means for illuminating only a vicinity of the bottom portion of the imaging vessel by the excitation light, and means for obtaining images from below the bottom portion of the imaging vessel.

[0006] In accordance with this arrangement, as the fluorescent particles that are the object of interest are accumulated in the bottom part of the imaging vessel and the excitation light is projected on just that bottom part of the vessel, it is possible to reduce background

light and thereby improve the contrast of the images obtained.

[0007] As a means that can be used to ensure that just the bottom portion of the vessel is illuminated, a cover can be positioned to prevent the upper part of the imaging vessel from being illuminated by the excitation light, or the same effect can be obtained by using a cover with a slit-shaped aperture.

[0008] In such a case, the fluorescent particles can be illuminated even more effectively by using a cylindrical lens or the like to convert the thin excitation laser beam into a wide, flat beam in order to illuminate just the bottom portion of the vessel. The same effect can also be obtained by deflecting the excitation light along the bottom portion, or by projecting the excitation light via a bundle of optical fibers the exit end of which is arranged in a straight line.

[0009] Thus markedly reducing the amount of background light allows the fluorescent particles to be imaged with high contrast, thereby increasing the reliability of the fluorescent particle count.

[0010] Further features of the invention, its nature and various advantages will become more apparent from the accompanying drawings and following detailed description of the invention.

[0011] Figure 1 is a block diagram showing the general configuration of an apparatus for imaging fluorescent particles according to the present invention.

[0012] Figure 2 is a front view of the apparatus used for analyzing and displaying obtained fluorescent particle images.

[0013] Figure 3 is a diagram illustrating an arrangement of a cover used to shield the upper part of the imaging vessel.

[0014] Figure 4 is a diagram illustrating another arrangement for shielding the upper part of the imaging vessel from illuminating light.

[0015] Figure 5 is a diagram of an optical system used to form a strip-shaped excitation light beam.

[0016] Figure 6 is a diagram of another optical system used to form a strip-shaped excitation light beam.

[0017] Figure 7 is a diagram showing the configuration of optical elements used to form a strip-shaped excitation light beam.

[0018] Figure 8a is an exterior perspective view of the imaging vessel.

[0019] Figure 8b is a cross-sectional view of the vessel.

[0020] Figure 8c is a bottom view of the vessel.

[0021] Figure 9 is a cross-sectional view of an imaging vessel according to another configuration.

[0022] Figure 10 is a cross-sectional horizontal view of an imaging vessel according to yet another configuration.

[0023] Figures 1 and 2 show the arrangement of a first embodiment of the present invention. In the drawings, reference numeral 1 denotes a laser light source, such as, for example, a YAG laser that produces a green laser

beam. The laser beam from the laser light source 1 impinges upon, and is diffused by, a diffusion plate 2 comprised of ground glass or other such member that is able to diffuse light. The light thus diffused is projected at a bottom portion 3' of an imaging vessel 3, the upper part of which is covered by a cover 4. Fluorescent particles are accumulated in the bottom portion of the imaging vessel 3, and these fluorescent particles fluoresce when illuminated by the laser beam. The images of the fluorescent particles illuminated by the laser beam pass via a cover-glass 5 and objective lens 6 to a mirror 7 that reflects the images to a barrier filter 8 that transmits light in a prescribed frequency band, and are then picked up by a CCD camera 9.

[0024] The images of the fluorescent particles picked up by the CCD camera 9 are passed via a signal line 10 to a video capture device 11 of a computer 12, where they are processed by an image processing circuit 13 (Figure 2) to enable the fluorescent particles to be recognized. There is a change in brightness where there is a fluorescent particle, so the fluorescent particles can be recognized by, for example, using the differentiation of signal values to detect the positional coordinates of the particles. The fluorescent particles thus recognized are displayed on a monitor 14. Figure 2 depicts the image 15 of the bottom portion of the vessel together with a plurality of fluorescent particles 15a therein, displayed on the monitor 14. The fluorescent particles 15a are counted and the count is also displayed at the lower part 16 of the monitor 14.

[0025] The imaging vessel 3 is molded in one piece from transparent polystyrene resin, glass, or acrylic resin, preferably polystyrene resin. Inserted into the imaging vessel 3 are a platelet preparation sample (100 microliters, for example), a chemical (Triton X) that dissolves platelet and leukocyte cytoplasm, and a fluorescent dye (propidium iodide) for staining leukocyte nuclei. The imaging vessel 3 is then subjected to centrifugal separation in a centrifuge (not shown), causing the leukocyte nuclei to collect in the bottom portion of the imaging vessel 3. All of the leukocyte nuclei can be collected in the bottom portion 3' of the imaging vessel 3 by applying a prescribed centrifugal force.

[0026] The cover 4 is then used to cover the imaging vessel 3 in which the leukocyte nuclei stained with a fluorescent dye are collected in the bottom portion 3' thereof, and the imaging vessel 3 is mounted on the imaging apparatus. For imaging, the laser light source 1 is activated, producing a laser beam which is diffused by the diffusion plate 2 and projected onto the bottom portion 3' of the imaging vessel 3. As the nuclei of the leukocytes in the bottom portion 3' of the imaging vessel 3 have been stained with a fluorescent dye, when they are illuminated by the beam of excitation light, they emit fluorescent light having a frequency of around 600 nm. This is picked up via the cover-glass 5, objective lens 6, mirror 7 and barrier filter 8 below the imaging vessel 3. The barrier filter 8 only transmits light having the frequency

of fluorescent light, allowing light of harmful frequencies to be blocked at this point.

[0027] The laser beam is projected only at the bottom portion of the vessel, effectively illuminating the leukocytes collected there. Therefore, even if there is fluorescent dye floating in the solution in the imaging vessel 3, it is possible to prevent the fluorescent dye from forming harmful background light, thereby enabling the images to be obtained with improved contrast.

[0028] With reference to Figure 2, the images of fluorescent particles thus obtained by the CCD camera 9 are passed via a signal line 10 to a video capture device 11 of a computer 12, where they are processed by an image processing circuit 13 to count the number of leukocytes 15a.

[0029] In accordance with the arrangement described above, the laser beam is projected only onto the bottom portion of the imaging vessel and does not illuminate the upper part of the vessel. As shown by Figure 3, the effect of only illuminating the bottom portion can be enhanced by providing a cover 20 that shields all parts other than the bottom portion from the illuminating light beam.

[0030] Instead of the cover arrangement of Figure 3, an arrangement such as that shown in Figure 4 may be used. In this arrangement, a mask 21 having a central slit-shaped aperture 21a is used. The laser beam from the laser light source 1 passes through the aperture 21a, ensuring that only the bottom portion 3' of the imaging vessel 3 is illuminated.

[0031] A linear or line-shaped light beam would illuminate more of the bottom portion of the imaging vessel than a spot-shaped beam. Figure 5 shows the type of arrangement that could be used in such a case, with laser beam 30 being shaped to a flat beam 33 by passage through cylindrical lenses 31 and 32, and the flat beam 33 being used to illuminate the bottom portion 3' of the imaging vessel 3.

[0032] Figure 6 shows another arrangement, in which a scanning mirror 40 is used to deflect the laser beam 30, which passes through a lens 41 to scan the bottom portion of the imaging vessel.

[0033] Figure 7 shows another arrangement, that uses a bundle of optical fibers. In this arrangement, the fibers at the entrance end 50 from which the laser beam enters are arranged in a round configuration, while at the exit end 51 the fibers are arranged in a straight line, with the exit end 51 being disposed in the vicinity of the bottom portion of the imaging vessel so as to illuminate the bottom portion thereof.

[0034] In each of these embodiments, only the bottom portion of the vessel, or the vicinity thereof, is illuminated, by a strip-shaped beam, thereby making it possible to obtain images of the fluorescent particles with good contrast. Moreover, if a diffusion plate 2 is used to diffuse the laser beam, it enables the bottom portion of the imaging vessel to be illuminated uniformly.

[0035] Figure 8 shows a preferred embodiment of the imaging vessel 3, preferably formed as a one-piece

molding of polystyrene resin. The vessel has a ring-shaped upper portion 3a having a notch 3f for positioning purposes. The imaging vessel 3 comprises a cylindrical portion 3b that extends vertically downward from the upper portion 3a to a small-diameter portion 3d, via a sloping portion 3c. A substantially square or rectangular block portion 3e is formed at the lower end. One side of the block portion 3e is arranged to be illuminated by a laser beam, indicated by the arrow. When fluorescent particles are to be imaged, the imaging vessel 3 is attached to the apparatus, with the notch 3f being used to position the imaging vessel 3. When the vessel 3 has been fitted into position, a flat face of the block portion 3e is perpendicular to the direction of laser beam illumination, forming the entry surface for the incident beam. Thus, the laser beam illuminates only the bottom portion 3g of the imaging vessel 3.

[0036] The bottom portion may also be illuminated by an arrangement such as the one shown in Figure 9, in which the block portion 3e has a round cross-section, and a negative cylindrical lens 60 is disposed on the side from which the bottom portion is illuminated by the excitation beam.

[0037] In the arrangement shown in Figure 10, a cover 70 is used to shield parts of the imaging vessel 3 other than the bottom portion from the laser beam. In this case, the cover 20, shown in Figure 3, on the imaging apparatus side may be omitted. The cover or shielding function may be realized by applying a light shield coating to the vessel, or by painting the vessel in a shielding color.

[0038] It is to be understood that while in the foregoing the invention has been described with reference to leukocytes as the fluorescent particles, the invention is not limited thereto but can be applied to other fluorescent particles.

[0039] As described in the foregoing, in accordance with the present invention, fluorescent particles to be imaged are collected in the bottom portion of an imaging vessel, only that bottom portion is illuminated by an excitation light beam, and the bottom portion is imaged from below. The result is that background light is reduced, making it possible to obtain high-contrast images of the fluorescent particles, thereby making it possible to evaluate the images of the fluorescent particles and count them with greater precision.

#### Claims

1. An apparatus for imaging fluorescent particles stained with a fluorescent dye, comprising:

an imaging vessel (3) for collecting and accommodating the fluorescent particles in a bottom portion (3') thereof;  
means (1) for generating an excitation light for exciting the fluorescent particles;

illumination means (2,5,7,8) for illuminating only a vicinity of the bottom portion of the imaging vessel by the excitation light; and  
means (9) for obtaining images from below the bottom portion of the imaging vessel.

2. An apparatus according to claim 1, wherein parts of the imaging vessel other than the bottom portion (3') are covered by shielding (20).
3. An apparatus according to claim 1, further comprising a mask (21) having a slit-shaped aperture (21a), wherein only a vicinity of the bottom portion of the imaging vessel is illuminated by excitation light passing through the slit-shaped aperture.
4. An apparatus according to any of claims 1 to 3, wherein the illumination means include a cylindrical lens (31, 32) that forms the excitation light into a flat beam (33) for illuminating only a vicinity of the bottom portion of the imaging vessel.
5. An apparatus according to any of claims 1 to 3, wherein the illumination means include a scanning means (40) for deflecting the excitation light along a vicinity of the bottom portion of the imaging vessel.
6. An apparatus according to any of claims 1 to 3, wherein the illumination means include a bundle of optical fibers, the exit end (51) of which is arranged in a straight line for illuminating only a vicinity of the bottom portion of the imaging vessel.
7. An apparatus according to any of claims 1 to 6, wherein the illumination means include an optical element (2) for uniformly illuminating a vicinity of the bottom portion of the imaging vessel.
8. An apparatus according to claim 7, wherein the optical element is a diffusion plate (2).
9. An apparatus according to any of claims 1 to 8, wherein the excitation light is a laser beam.
10. An apparatus according to any of claims 1 to 9, wherein part (3e) of side surfaces of the bottom portion of the imaging vessel forms an excitation light entry surface.
11. An apparatus according to claim 10, wherein the bottom portion of the imaging vessel has a square or rectangular horizontal cross-section (3e) and one side thereof forms an excitation light entry surface.
12. An apparatus according to claim 10, wherein the bottom portion of the imaging vessel has a round horizontal cross-section (3e) and a negative cylindrical lens (60) is disposed on the side from which

the bottom portion is illuminated by the excitation light.

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FIG. 1

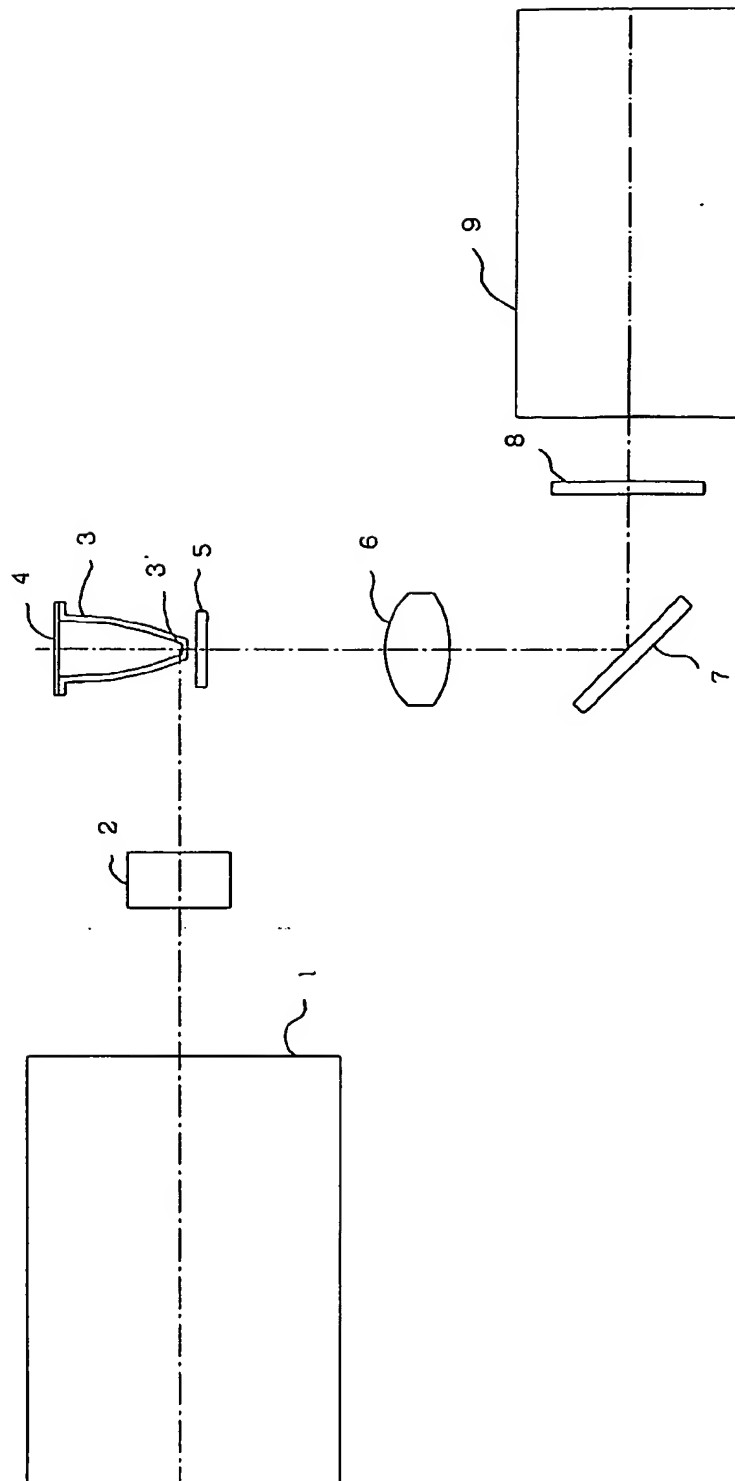


FIG. 2

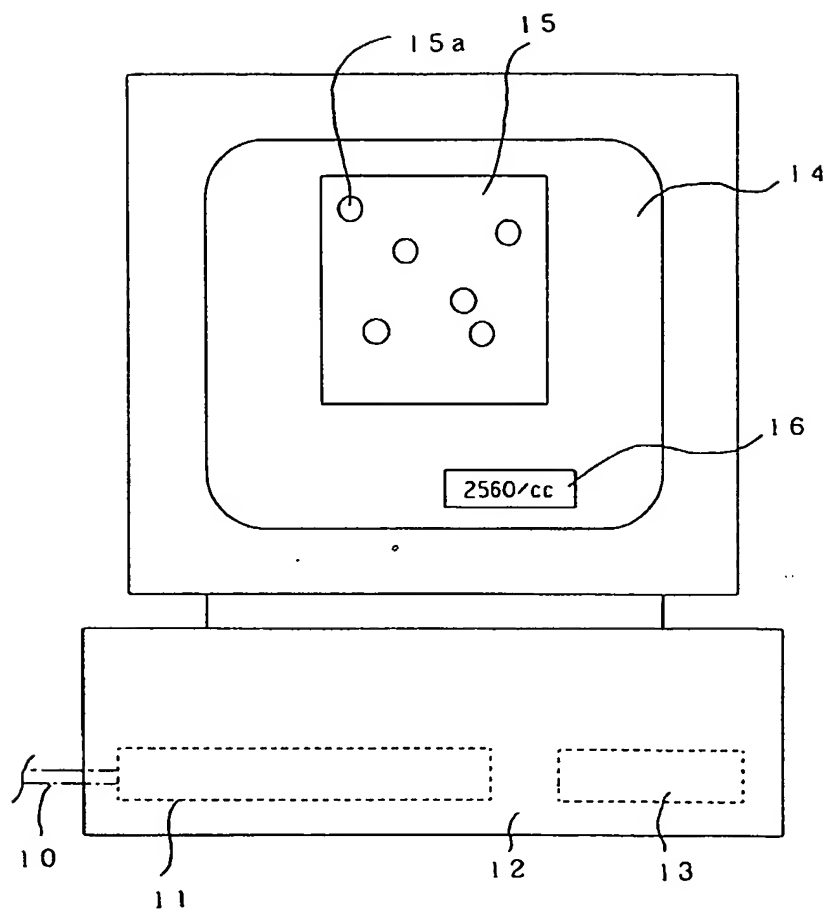
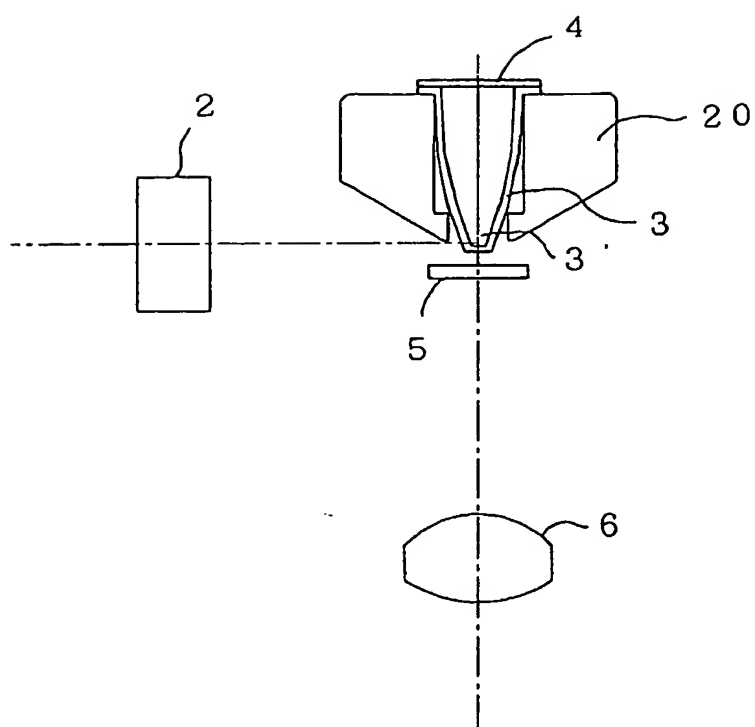
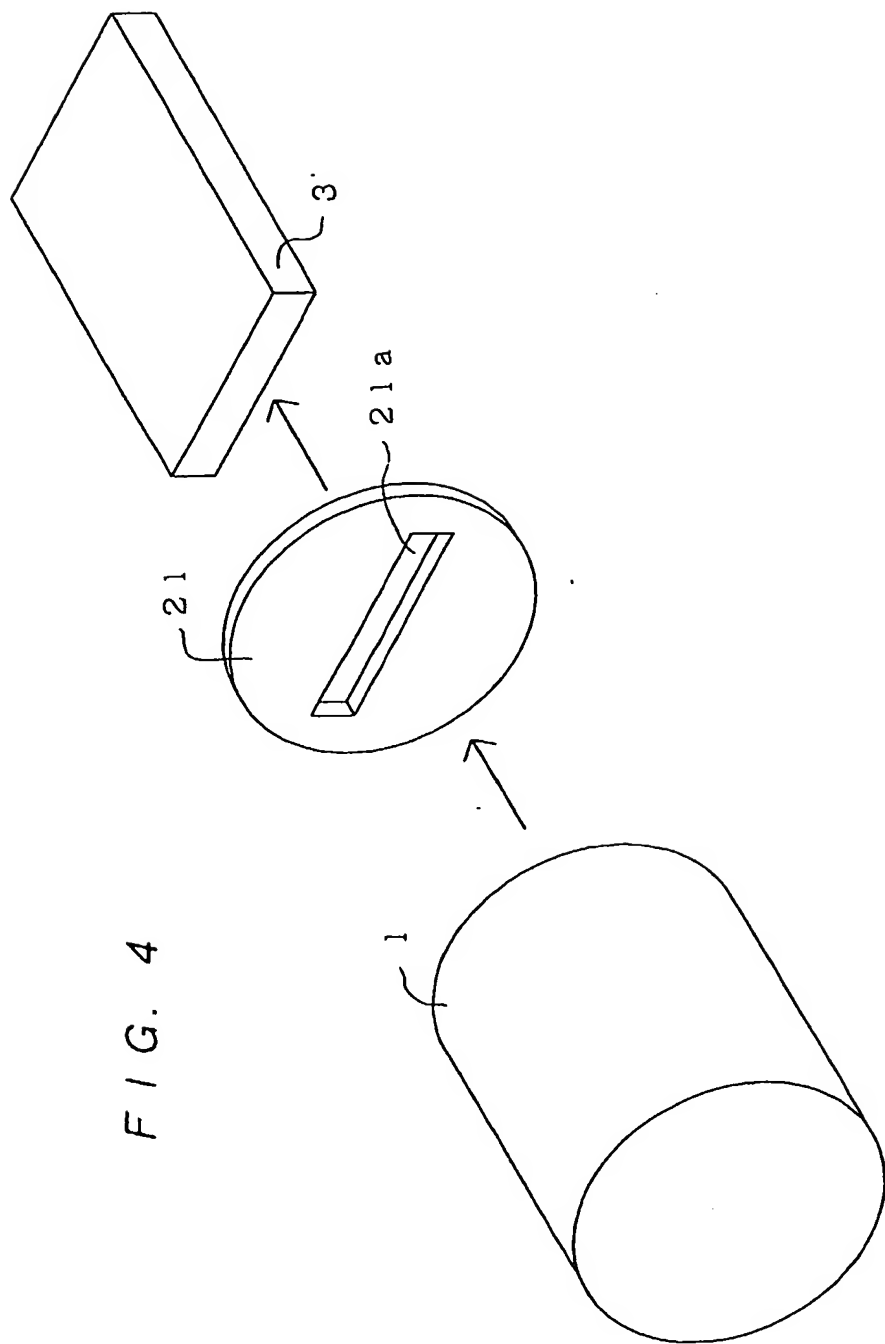
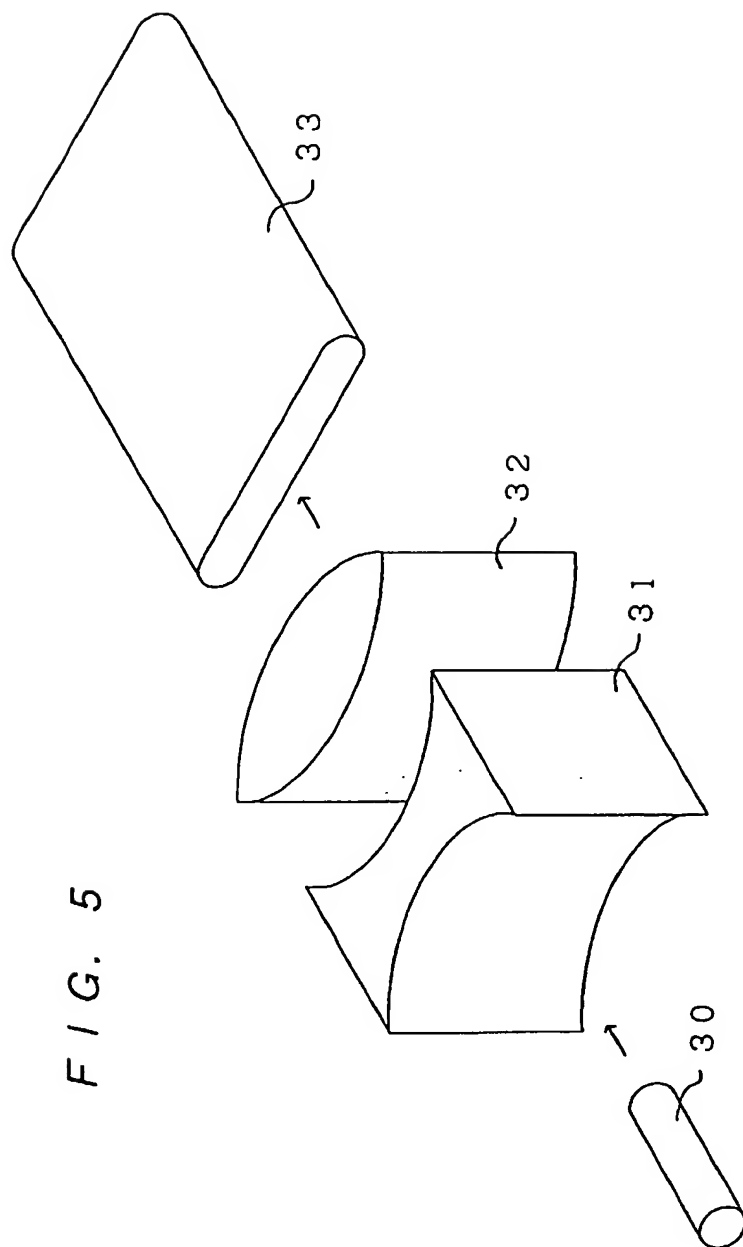


FIG. 3









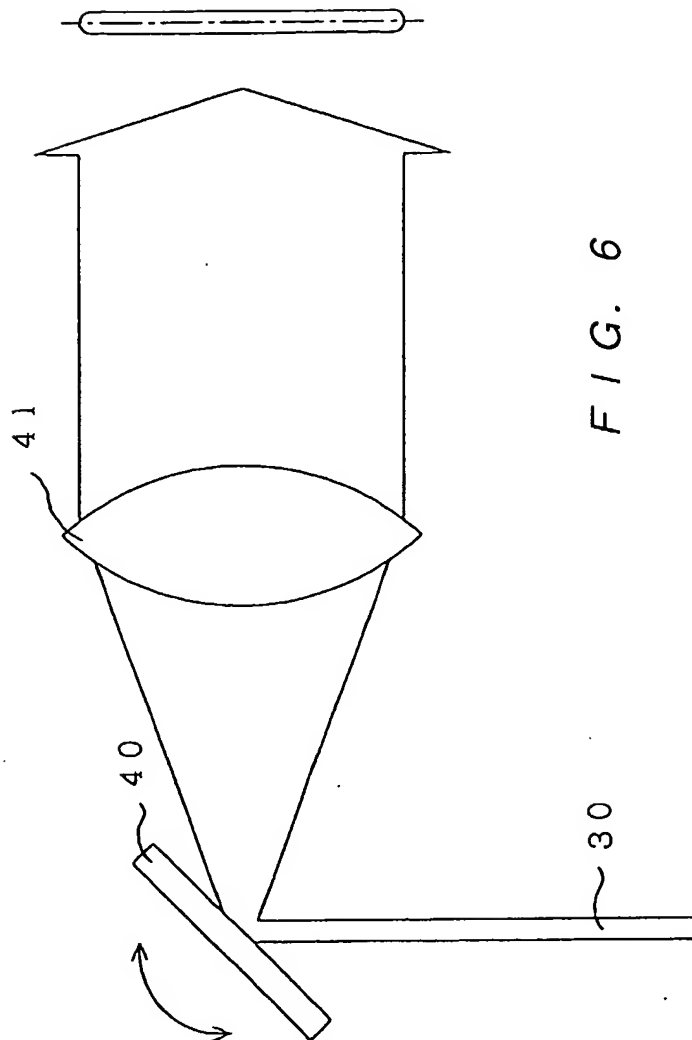
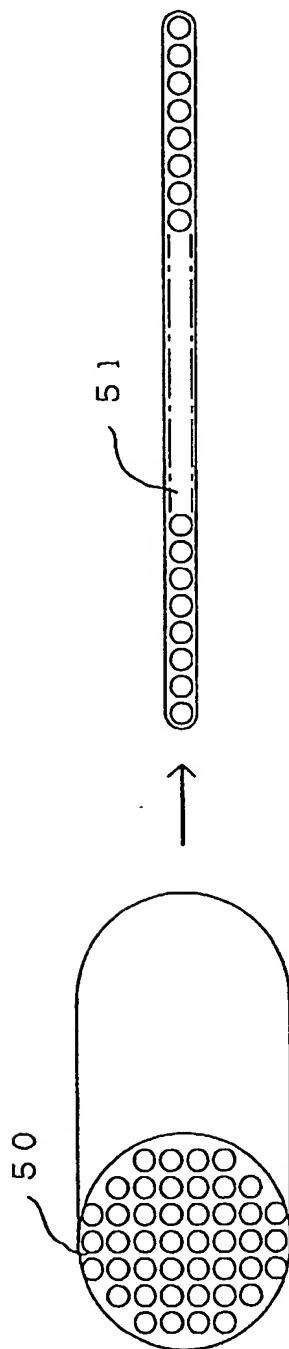


FIG. 7



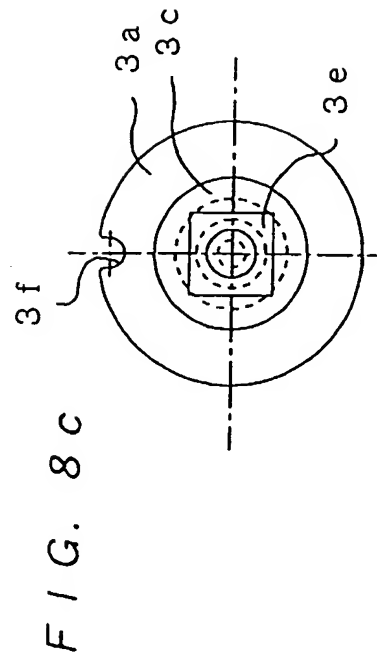
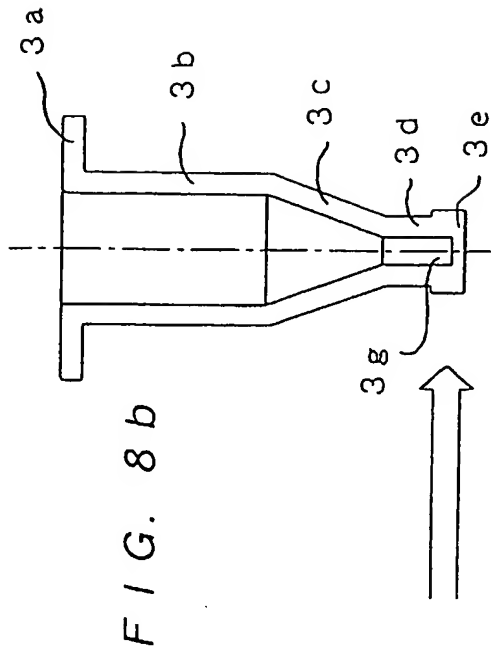
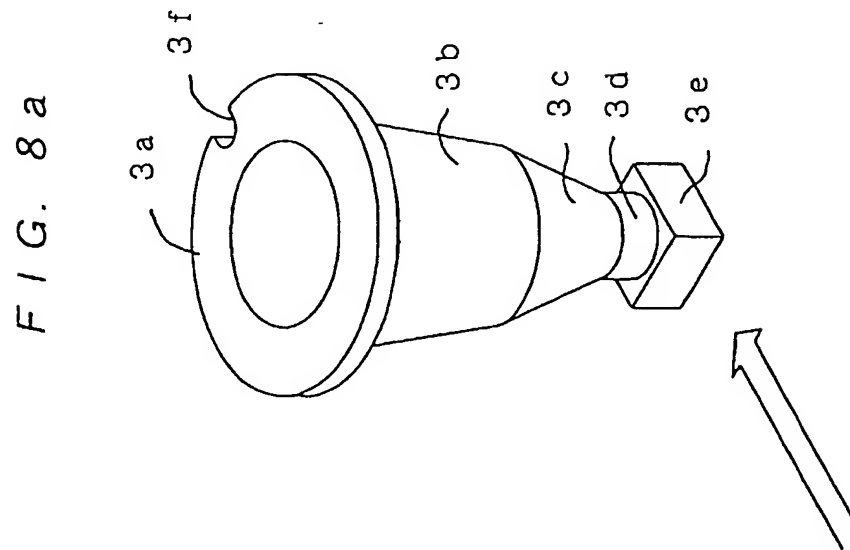


FIG. 9

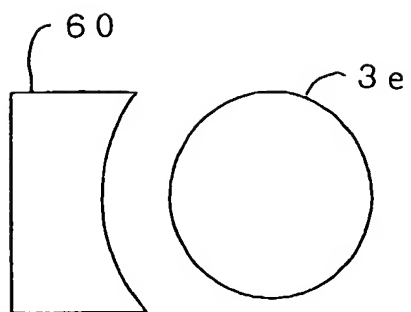


FIG. 10

